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FILE COVERS 1907 - 15 Apr 2003 VOL 138 ISS 16
FILE LAST UPDATED: 14 Apr 2003 (20030414/ED)

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L3	938	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	M2-1
L4	2203	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	(RESPIRATORY SYNCYTIAL OR RSV)
			(W) VIRUS			
L7	23	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	L4 AND L3

L3	938	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	M2-1
L4	2203	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	(RESPIRATORY SYNCYTIAL OR RSV)
			(W) VIRUS			
L8	250532	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GLYCINE OR VALINE OR ASPARTIC
			ACID OR ALANINE			
L20	2	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	L4 AND L8 AND L3

L3	938	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	M2-1
L8	250532	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GLYCINE OR VALINE OR ASPARTIC
			ACID OR ALANINE			
L10	105222	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GENE, MICROBIAL/CT
L11	120756	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	MUTAGENESIS/CT OR MUTATION/CW
L12	15815	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GENETIC VECTORS/CT
L13	4211	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	VIRAL VECTORS/CT
L14	5359	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	VIRUS VECTORS/CT
L15	32535	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	VACCINES/CT
L16	33736	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	IMMUNITY/CT
L21	0	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	L3 AND L8 AND (L10 OR L11 OR
						L12 OR L13 OR L14) AND (L15 OR L16)

L3	938	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	M2-1
L8	250532	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GLYCINE OR VALINE OR ASPARTIC
			ACID OR ALANINE			
L10	105222	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	GENE, MICROBIAL/CT
L11	120756	SEA	FILE=CAPLUS	ABB=ON	PLU=ON	MUTAGENESIS/CT OR MUTATION/CW

L12 15815 SEA FILE=CAPLUS ABB=ON PLU=ON GENETIC VECTORS/CT
L13 4211 SEA FILE=CAPLUS ABB=ON PLU=ON VIRAL VECTORS/CT
L14 5359 SEA FILE=CAPLUS ABB=ON PLU=ON VIRUS VECTORS/CT
L15 32535 SEA FILE=CAPLUS ABB=ON PLU=ON VACCINES/CT
L16 33736 SEA FILE=CAPLUS ABB=ON PLU=ON IMMUNITY/CT
L22 1 SEA FILE=CAPLUS ABB=ON PLU=ON L3 AND L8 AND (L10 OR L11 OR
L12 OR L13 OR L14 OR L15 OR L16)

=> s 17 or 120 or 122

L58 23 L7 OR L20 OR L22

=> file medline; d que 136

FILE 'MEDLINE' ENTERED AT 18:07:36 ON 15 APR 2003

FILE LAST UPDATED: 10 APR 2003 (20030410/UP). FILE COVERS 1958 TO DATE.

On April 13, 2003, MEDLINE was reloaded. See HELP RLOAD for details.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2003 vocabulary. See <http://www.nlm.nih.gov/mesh/changes2003.html> for a description on changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

L26 50 SEA FILE=MEDLINE ABB=ON PLU=ON RESPIRATORY SYNCYTIAL VIRUS
VACCINES/CT
L27 3856 SEA FILE=MEDLINE ABB=ON PLU=ON RESPIRATORY SYNCYTIAL
VIRUSES+NT/CT
L28 93765 SEA FILE=MEDLINE ABB=ON PLU=ON MUTAGENESIS+NT/CT
L29 31841 SEA FILE=MEDLINE ABB=ON PLU=ON GENETIC VECTORS+NT/CT
L30 294933 SEA FILE=MEDLINE ABB=ON PLU=ON MUTATION+NT/CT
L31 6829 SEA FILE=MEDLINE ABB=ON PLU=ON GENETICS, MICROBIAL+NT/CT
L35 286 SEA FILE=MEDLINE ABB=ON PLU=ON M2 1
L36 7 SEA FILE=MEDLINE ABB=ON PLU=ON (L26 OR L27) AND (L28 OR L29
OR L30 OR L31) AND L35

=> file embase; d que 145

FILE 'EMBASE' ENTERED AT 18:07:43 ON 15 APR 2003

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FILE COVERS 1974 TO 10 Apr 2003 (20030410/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

L37 4365 SEA FILE=EMBASE ABB=ON PLU=ON RESPIRATORY SYNCYTIAL PNEUMOVIR
US/CT
L38 185 SEA FILE=EMBASE ABB=ON PLU=ON M2 1
L40 17109 SEA FILE=EMBASE ABB=ON PLU=ON MUTAGENESIS/CT
L41 761 SEA FILE=EMBASE ABB=ON PLU=ON DNA VECTOR/CT
L42 221181 SEA FILE=EMBASE ABB=ON PLU=ON MUTATION+NT/CT
L43 9353 SEA FILE=EMBASE ABB=ON PLU=ON VIRUS VECTOR+NT/CT
L44 129618 SEA FILE=EMBASE ABB=ON PLU=ON MICROBIAL GENETICS+NT/CT
L45 16 SEA FILE=EMBASE ABB=ON PLU=ON L37 AND L38 AND (L40 OR L41 OR

L42 OR L43 OR L44)

=> file wpid; d que 155; d que 156
FILE 'WPIDS' ENTERED AT 18:07:53 ON 15 APR 2003
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FILE LAST UPDATED: 10 APR 2003 <20030410/UP>
MOST RECENT DERWENT UPDATE: 200324 <200324/DW>
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L49	447	SEA FILE=WPIDS ABB=ON	PLU=ON	M2 1
L52	17435	SEA FILE=WPIDS ABB=ON	PLU=ON	VACCIN?
L54	3	SEA FILE=WPIDS ABB=ON	PLU=ON	L49 AND L52
L55	2	SEA FILE=WPIDS ABB=ON	PLU=ON	L54 NOT NITROOXY/TI

L49	447	SEA FILE=WPIDS ABB=ON	PLU=ON	M2 1
L50	520	SEA FILE=WPIDS ABB=ON	PLU=ON	(RESPIRATORY SYNCYTIAL OR RSV) (W) VIRUS
L51	3	SEA FILE=WPIDS ABB=ON	PLU=ON	L49 AND L50
L56	2	SEA FILE=WPIDS ABB=ON	PLU=ON	L51 NOT SAMPLE/TI

=> s 155 or 156
L59 2 L55 OR L56

=> dup rem 136 158 145 159
FILE 'MEDLINE' ENTERED AT 18:08:45 ON 15 APR 2003

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PROCESSING COMPLETED FOR L58
PROCESSING COMPLETED FOR L45
PROCESSING COMPLETED FOR L59
L60 24 DUP REM L36 L58 L45 L59 (24 DUPLICATES REMOVED)
ANSWERS '1-7' FROM FILE MEDLINE

ANSWERS '8-23' FROM FILE CAPLUS
ANSWER '24' FROM FILE WPIDS

=> d ibib ab 160 1-24

L60 ANSWER 1 OF 24 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002131185 MEDLINE
DOCUMENT NUMBER: 21851099 PubMed ID: 11861854
TITLE: Identification of temperature-sensitive mutations in the phosphoprotein of respiratory syncytial virus that are likely involved in its interaction with the nucleoprotein.
AUTHOR: Lu Bin; Brazas Robert; Ma Chien-Hui; Kristoff Tina; Cheng Xing; Jin Hong
CORPORATE SOURCE: Aviron, Mountain View, California 94043, USA:
CONTRACT NUMBER: 2R44A145267-01/02
SOURCE: JOURNAL OF VIROLOGY, (2002 Mar) 76 (6) 2871-80.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020228
Last Updated on STN: 20020403
Entered Medline: 20020329
AB The phosphoprotein (P) of human respiratory syncytial virus (RSV) is an essential component of the viral RNA polymerase, along with the large polymerase (L), nucleocapsid (N), and M2-1 proteins. By screening a randomly mutagenized P gene cDNA library, two independent mutations, one with a substitution of glycine at position 172 by serine (G172S) and the other with a substitution of glutamic acid at position 176 by glycine (E176G), were identified to result in the loss of N-P interaction at 37 degrees C in the yeast two-hybrid assay. Both P mutants exhibited greatly reduced activity in supporting the replication and transcription of an RSV minigenome replicon at 37 and 39 degrees C. The G172S and E176G mutations were introduced individually into the RSV A2 (rA2) antigenomic cDNA, and recombinant viruses, rA2-P172 and rA2-P176, were obtained. Both viruses replicate as well as wild-type A2 virus in both Vero and HEp-2 cells at 33 degrees C, but each mutant virus exhibited temperature-sensitive replication in both cell lines. rA2-P176 is more temperature sensitive than rA2-P172. Coimmunoprecipitation of the N protein with each P mutant from virus-infected cells demonstrates that N-P interaction is impaired at 37 degrees C. In addition, the levels of replication of rA2-P172 and rA2-P176 in the lungs of mice and cotton rats were reduced. As is the case with the in vitro assays, rA2-P176 is more restricted in replication in the lower respiratory tract of mice and cotton rats than rA2-P172. During in vitro passage at 37 degrees C, the E176G mutation in rA2-P176 was rapidly changed from glycine to predominantly aspartic acid; mutations to cysteine or serine were also detected. All of the revertants lost the temperature-sensitive phenotype. To analyze the importance of the amino acids in the region from positions 161 to 180 for the P protein function, additional mutations were introduced and their functions were analyzed in vitro. A double mutant containing both G172S and E176G changes in the P gene, substitution of the three charged residues at positions 174 to 176 by alanine, and a deletion of residues from positions 161 to 180 completely abolished the P protein function in the minigenome assay. Thus, the amino acids at positions 172 and 176 and the adjacent charged residues play critical roles in the function of the P protein.

L60 ANSWER 2 OF 24 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 2001639952 MEDLINE
DOCUMENT NUMBER: 21548262 PubMed ID: 11689613
TITLE: Requirement of cysteines and length of the human
respiratory syncytial virus **M2-1**
protein for protein function and virus viability.
AUTHOR: Tang R S; Nguyen N; Cheng X; Jin H
CORPORATE SOURCE: Aviron, Mountain View, California 94043, USA.
CONTRACT NUMBER: 2R44A145267-01/02
SOURCE: JOURNAL OF VIROLOGY, (2001 Dec) 75 (23) 11328-35.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011107
Last Updated on STN: 20020123
Entered Medline: 20011207

AB The **M2-1** protein of human respiratory syncytial virus (hRSV) promotes processive RNA synthesis and readthrough at RSV gene junctions. It contains four highly conserved cysteines, three of which are located in the Cys(3)-His(1) motif at the N terminus of **M2-1**. Each of the four cysteines, at positions 7, 15, 21, and 96, in the **M2-1** protein of hRSV A2 strain was individually replaced by glycines. When tested in an RSV minigenome replicon system using beta-galactosidase as a reporter gene, C7G, C15G, and C21G located in the Cys(3)-His(1) motif showed a significant reduction in processive RNA synthesis compared to wild-type (wt) **M2-1**. C96G, which lies outside the Cys(3)-His(1) motif, was fully functional in supporting processive RNA synthesis in vitro. Each of these cysteine substitutions was introduced into an infectious antigenomic cDNA clone derived from hRSV A2 strain. Except for C96G, which resulted in a viable virus, no viruses were recovered with mutations in the Cys(3)-His(1) motif. This indicates that the Cys(3)-His(1) motif is critical for **M2-1** function and for RSV replication. The functional requirement of the C terminus of the **M2-1** protein was examined by engineering premature stop codons that caused truncations of 17, 46, or 67 amino acids from the C terminus. A deletion of 46 or 67 amino acids abolished the synthesis of full-length beta-galactosidase mRNA and did not result in the recovery of viable viruses. However, a deletion of 17 amino acids from the C terminus of **M2-1** reduced processive RNA synthesis in vitro and was well tolerated by RSV. Relocation of the **M2-1** termination codon upstream of the **M2-2** initiation codons did not significantly affect the expression of the **M2-2** protein. Both rA2-Tr17 and rA2-C96G did not replicate as efficiently as wt rA2 in HEp-2 cells and was restricted in replication in the respiratory tracts of cotton rats.

L60 ANSWER 3 OF 24 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2001556229 MEDLINE
DOCUMENT NUMBER: 21488914 PubMed ID: 11601901
TITLE: Synergistic effects of gene-end signal mutations and the
M2-1 protein on transcription termination
by respiratory syncytial virus.
AUTHOR: Sutherland K A; Collins P L; Peeples M E
CORPORATE SOURCE: Department of Immunology/Microbiology, Rush-Presbyterian-
St. Luke's Medical Center, 1653 W. Congress Parkway,
Chicago, Illinois 60612, USA.
CONTRACT NUMBER: AI47213 (NIAID)
SOURCE: VIROLOGY, (2001 Sep 30) 288 (2) 295-307.
Journal code: 0110674. ISSN: 0042-6822.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011017
Last Updated on STN: 20020122
Entered Medline: 20011204

AB Individual mononegavirus genes terminate with a short cis-acting element, the gene-end (GE) signal, that directs polyadenylation and termination and might also influence the efficiency of reinitiation at the next downstream gene. The 12-13 nucleotide (nt) GE signals of human respiratory syncytial virus (RSV) consist of a conserved pentanucleotide (3'-UCAAU, negative sense), followed by a 3-nt middle region that is AU-rich but otherwise not conserved, followed by a 4- or 5-nt poly(U) region that is thought to generate the poly(A) tail of the encoded mRNA by reiterative copying. Most of the naturally occurring differences in the GE signals of the various RSV genes occur in the "middle" and "poly(U)" regions. We mutated a copy of the fusion protein (F) GE signal that was positioned at the end of the promoter-proximal gene of a tricistronic minigenome and evaluated the effect of these mutations on RSV transcription in a plasmid-initiated, intracellular assay. Mutations confirmed the importance of the middle region's AU-rich nature and 3-nt length, and the poly(U) tract's 4-nt minimum functional length, with maximal termination efficiency observed at five U residues. Nt assignments other than U at position 13 also affected the efficiency of termination, showing that this position is part of the functional 13-nt GE signal. These results indicate that differences in nt assignments in the middle and poly(U) regions of the GE signal, which occur frequently in nature, affect the efficiency of termination. Unexpectedly, the ability of certain mutations to inhibit termination was completely dependent on coexpression of the **M2-1** protein, and in many other cases the inhibitory effect of the mutation was greatly enhanced in the presence of **M2-1**. Thus, **M2-1** appears to have the effect of altering the polymerase such that it ignores suboptimal GE signals. Interestingly, certain mutations that greatly decreased the efficiency of termination in the absence of **M2-1** did not have much effect on the expression of the second gene, implying that correct termination and/or polyadenylation at the upstream gene is not obligatory for reinitiation at the next downstream gene.
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L60 ANSWER 4 OF 24 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 2001031484 MEDLINE
DOCUMENT NUMBER: 20481623 PubMed ID: 11024112
TITLE: Structural phosphoprotein **M2-1** of the human respiratory syncytial virus is an RNA binding protein.
AUTHOR: Cuesta I; Geng X; Asenjo A; Villanueva N
CORPORATE SOURCE: Centro Nacional de Microbiologia, Instituto de Salud Carlos III, Majadahonda, Madrid 28220, Spain.
SOURCE: JOURNAL OF VIROLOGY, (2000 Nov) 74 (21) 9858-67.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001120

AB The structural phosphoprotein **M2-1** of human respiratory syncytial virus (HRSV) Long strain shows RNA binding capacity in three different assays that detect RNA-protein complexes: cross-linking, gel retardation, and Northern-Western assays. It is able to bind HRSV leader RNA specifically with cooperative kinetics, with an apparent $K(d)$ of at least 90 nM. It also binds to long RNAs with no sequence specificity. The RNA binding domain has been located between amino acid residues 59 and 85, at the NH(2) terminus of the protein. This region contains the phosphorylatable amino acid residues threonine 56 and serine 58, whose modification decreases the binding capacity of **M2-1** protein to long RNAs.

L60 ANSWER 5 OF 24 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 2000387825 MEDLINE
DOCUMENT NUMBER: 20304979 PubMed ID: 10846068
TITLE: The Cys(3)-His(1) motif of the respiratory syncytial virus **M2-1** protein is essential for protein function.
AUTHOR: Hardy R W; Wertz G W
CORPORATE SOURCE: Department of Microbiology, University of Alabama School of Medicine at Birmingham, 35294, USA.
CONTRACT NUMBER: AI12464 (NIAID)
AI20181 (NIAID)
SOURCE: JOURNAL OF VIROLOGY, (2000 Jul) 74 (13) 5880-5.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000810

AB The M2 gene of respiratory syncytial (RS) virus has two open reading frames (ORFs). ORF1 encodes a 22-kDa protein termed **M2-1**. The **M2-1** protein contains a Cys(3)-His(1) motif (C-X(7)-C-X(5)-C-X(3)-H) near the amino terminus. This motif is conserved in all human, bovine, and ovine strains of RS virus. A similar motif found in the mammalian transcription factor Nup475 has been shown to bind zinc. The **M2-1** protein of human RS virus functions as a transcription factor which increases polymerase processivity, and it enhances readthrough of intergenic junctions during RS virus transcription, thereby acting as a transcription antiterminator. The **M2-1** protein also interacts with the nucleocapsid protein. We examined the effects of mutations of cysteine and histidine residues predicted to coordinate zinc in the Cys(3)-His(1) motif on transcription antitermination and N protein binding. We found that mutating the predicted zinc-coordinating residues, the cysteine residues at amino acid positions 7 and 15 and the histidine residue at position 25, prevented **M2-1** from enhancing transcriptional readthrough. In contrast, mutations of amino acids within this motif not predicted to coordinate zinc had no effect. Mutations of the predicted zinc-coordinating residues in the Cys(3)-His(1) motif also prevented **M2-1** from interacting with the nucleocapsid protein. One mutation of a noncoordinating residue in the motif which did not affect readthrough during transcription, E10G, prevented interaction with the nucleocapsid protein. This suggests that **M2-1** does not require interaction with the nucleocapsid protein in order to function during transcription. Analysis of the **M2-1** protein in reducing sodium dodecyl sulfate-polyacrylamide gels revealed two major forms distinguished by their mobilities. The slower migrating

form was shown to be phosphorylated, whereas the faster migrating form was not. Mutations in the Cys(3)-His(1) motif caused a change in distribution of the **M2-1** protein from the slower to the faster migrating form. The data presented here show that the Cys(3)-His(1) motif of **M2-1** is essential for maintaining the functional integrity of the protein.

L60 ANSWER 6 OF 24 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 1999432220 MEDLINE
DOCUMENT NUMBER: 99432220 PubMed ID: 10500164
TITLE: The M2-2 protein of human respiratory syncytial virus is a regulatory factor involved in the balance between RNA replication and transcription.
AUTHOR: Bermingham A; Collins P L
CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, 7 Center Drive MSC 0720, Bethesda, MD 20892-0720, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Sep 28) 96 (20) 11259-64. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991101
Last Updated on STN: 19991101
Entered Medline: 19991021

AB The M2 mRNA of human respiratory syncytial virus (RSV) contains two overlapping ORFs, encoding the transcription antitermination protein (**M2-1**) and the 90-aa M2-2 protein of unknown function. Viable recombinant RSV was recovered in which expression of M2-2 was ablated, identifying it as an accessory factor dispensable for growth in vitro. Virus lacking M2-2 grew less efficiently than did the wild-type parent in vitro, with titers that were reduced 1, 000-fold during the initial 2-5 days and 10-fold by days 7-8. Compared with wild-type virus, the intracellular accumulation of RNA by M2-2 knockout virus was reduced 3- to 4-fold or more for genomic RNA and increased 2- to 4-fold or more for mRNA. Synthesis of the F and G glycoproteins, the major RSV neutralization and protective antigens, was increased in proportion with that of mRNA. In cells infected with wild-type RSV, mRNA accumulation increased dramatically up to approximately 12-15 hr after infection and then leveled off, whereas accumulation continued to increase in cells infected with the M2-2 knockout viruses. These findings suggest that M2-2 mediates a regulatory "switch" from transcription to RNA replication, one that provides an initial high level of mRNA synthesis followed by a shift in the RNA synthetic program in favor of genomic RNA for virion assembly. With regard to vaccine development, the M2-2 knockout has a highly desirable phenotype in which virus growth is attenuated while gene expression is concomitantly increased.

L60 ANSWER 7 OF 24 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 1998105793 MEDLINE
DOCUMENT NUMBER: 98105793 PubMed ID: 9445048
TITLE: The NS1 protein of human respiratory syncytial virus is a potent inhibitor of minigenome transcription and RNA replication.
AUTHOR: Atreya P L; Peebles M E; Collins P L
CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-0720, USA.

SOURCE: JOURNAL OF VIROLOGY, (1998 Feb) 72 (2) 1452-61.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 19980226
Entered Medline: 19980218

AB The NS1 protein (139 amino acids) is one of the two nonstructural proteins of human respiratory syncytial virus (RSV) and is encoded by a very abundant mRNA transcribed from the promoter-proximal RSV gene. The function of NS1 was unknown and was investigated here by using a reconstituted transcription and RNA replication system that involves a minireplicon and viral proteins (N, P, L and M2-1) expressed from separate cotransfected plasmids. Coexpression of the NS1 cDNA strongly inhibited transcription and RNA replication mediated by the RSV polymerase, even when the level of expressed NS1 protein was substantially below that observed in RSV-infected cells. The effect depended on synthesis of NS1 protein rather than NS1 RNA alone. Transcription and both steps of RNA replication, namely, synthesis of the antigenome and the genome, appeared to be equally sensitive to inhibition. The efficiency of encapsidation of the plasmid-derived minigenome was not altered by coexpression of NS1, indicating that the inhibition occurs at a later step. In two different dicistronic minigenomes, transcription of each gene was equally sensitive to inhibition by NS1. This suggested that the gradient of transcriptional polarity was unaffected and that the effect of NS1 instead probably involves an early event such as polymerase entry on the genome. NS1-mediated inhibition of transcription and RNA replication was not affected by coexpression of the M2 mRNA, which has two open reading frames encoding the transcriptional elongation factor M2-1 and the putative negative regulatory factor M2-2. The potent nature of the NS1-mediated inhibition suggests that negative regulation is an authentic function of the NS1 protein, albeit not necessarily the only one.

L60 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:869921 CAPLUS
DOCUMENT NUMBER: 138:135888
TITLE: Recombinant **Respiratory syncytial virus** with the G and F genes shifted to the promoter-proximal positions
AUTHOR(S): Kreml, Christine; Murphy, Brian R.; Collins, Peter L.
CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, 20892-8007, USA
SOURCE: Journal of Virology (2002), 76(23), 11931-11942 .
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The genome of human **respiratory syncytial virus** (RSV) encodes 10 mRNAs and 11 proteins in the order 3'-NS1-NS2-N-P-M-SH-G-F-M2-1/M2-2-L-5'. The G and F glycoproteins are the major RSV neutralization and protective antigens. It seems likely that a high level of expression of G and F would be desirable for a live RSV vaccine. For mononegaviruses, the gene order is a major factor controlling the level of mRNA and protein expression due to the polar gradient of sequential transcription. In order to increase the expression of G and F, recombinant RSVs based on strain A2 were

constructed in which the G or F gene was shifted from the sixth or seventh position (in a genome lacking the SH gene), resp., to the first position (rRSV-G1/.DELTA.SH and rRSV-F1/.DELTA.SH, resp.). Another virus was made in which G and F were shifted together to the first and second positions, resp. (rRSV-G1F2/.DELTA.SH). Shifting one or two genes to the promoter-proximal position resulted in increased mRNA and protein expression of the shifted genes, with G and F expression increased up to 2.4- and 7.8-fold, resp., at the mRNA level and approx. 2.5-fold at the protein level, compared to the parental virus. Interestingly, the transcription of downstream genes was not greatly affected even though shifting G or F, or G and F together, had the consequence of moving the block of genes NS1-NS2-N-P-M-(G) one or two positions further from the promoter. The efficiency of replication of the gene shift viruses in vitro was increased up to 10-fold. However, their efficiency of replication in the lower respiratory tracts of mice was statistically indistinguishable from that of the parental virus. In the upper respiratory tract, replication was slightly reduced on some days for viruses in which G was in the first position. The magnitude of the G-specific antibody response to the gene shift viruses was similar to that to the parental virus, whereas the F-specific response was increased up to fourfold, although this was not reflected in an increase of the neutralizing activity. Thus, shifting the G and F genes to the promoter-proximal position increased virus replication in vitro, had little effect on replication in the mouse, and increased the antigen-specific immunogenicity of the virus beyond that of parental RSV.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 2002:275211 CAPLUS
DOCUMENT NUMBER: 137:17587
TITLE: **Respiratory syncytial virus** matrix protein associates with nucleocapsids in infected cells
AUTHOR(S): Ghildyal, R.; Mills, J.; Murray, M.; Vardaxis, N.; Meanger, J.
CORPORATE SOURCE: Children's Virology Research Unit, Macfarlane Burnet Institute for Medical Research and Public Health, Fairfield, 3078, Australia
SOURCE: Journal of General Virology (2002), 83(4), 753-757
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Little is known about the functions of the matrix (M) protein of **respiratory syncytial virus** (RSV). By analogy with other neg.-strand RNA viruses, the M protein should inhibit the viral polymerase prior to packaging and facilitate virion assembly. In this study, localization of the RSV M protein in infected cells and its assocn. with the RSV nucleocapsid complex was investigated. RSV-infected cells were shown to contain characteristic cytoplasmic inclusions. Further anal. showed that these inclusions were localization sites of the M protein as well as the N, P, L, and M2-1 proteins described previously. The M protein co-purified with viral ribonucleoproteins (RNPs) from RSV-infected cells. The transcriptase activity of purified RNPs was enhanced by treatment with antibodies to the M protein in a dose-dependent manner. These data suggest that the M protein is assocd. with RSV nucleocapsids and, like the matrix proteins of other neg.-strand RNA viruses, can inhibit virus transcription.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
ACCESSION NUMBER: 2001:100997 CAPLUS
DOCUMENT NUMBER: 134:161877
TITLE: Recombinant **RSV virus** expression
systems and vaccines
INVENTOR(S): Jin, Hong; Tang, Roderick; Li, Shengqiang; Bryant,
Martin
PATENT ASSIGNEE(S): Aviron, USA
SOURCE: PCT Int. Appl., 127 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001008703	A1	20010208	WO 2000-US21079	20000802
WO 2001008703	C2	20020808		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
EP 1204424	A1	20020515	EP 2000-952415	20000802
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL			

PRIORITY APPLN. INFO.: US 1999-368076 A 19990803
WO 2000-US21079 W 20000802

AB The present invention relates to genetically engineered recombinant **respiratory syncytial viruses** and viral vectors which contain deletions of various viral accessory gene(s) either singly or in combination. In accordance with the present invention, the recombinant respiratory syncytial viral vectors and viruses are engineered to contain complete deletions of the M2-2, NS1, NS2, or SH viral accessory genes or various combinations thereof. In addn., the present invention relates to the attenuation of **respiratory syncytial virus** by mutagenesis of the **M2-1** gene.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
ACCESSION NUMBER: 2001:888352 CAPLUS
DOCUMENT NUMBER: 136:131378
TITLE: **Respiratory syncytial virus M2-1** protein
requires phosphorylation for efficient function and binds viral RNA during infection
AUTHOR(S): Cartee, Tara L.; Wertz, Gail W.
CORPORATE SOURCE: Department of Microbiology, University of Alabama
School of Medicine, Birmingham, AL, 35294, USA
SOURCE: Journal of Virology (2001), 75(24), 12188-12197
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The **M2-1** protein of respiratory syncytial (RS) virus is a transcriptional processivity and antitermination factor. The **M2-1** protein has a Cys3His1 zinc binding motif which is essential for function, is phosphorylated, and has been shown to interact with the RS virus nucleocapsid (N) protein. In the work reported here, we detd. the sites at which the **M2-1** protein was phosphorylated and investigated the importance of these phosphorylated residues for **M2-1** function in transcription. By combining protease digestion, matrix-assisted laser desorption ionization-time of flight mass spectrometry, and site-directed mutagenesis, we identified the phosphorylated residues as serines 58 and 61, not threonine 56 and serine 58 as previously reported. Serines 58 and 61 and the surrounding amino acids are in a consensus sequence for phosphorylation by casein kinase I. Consistent with this, we showed that the unphosphorylated **M2-1** protein synthesized in *Escherichia coli* could be phosphorylated in vitro by casein kinase I. The effect of eliminating phosphorylation by site-specific mutagenesis of serines 58 and 61 on the function of the **M2-1** protein in transcription of RS virus subgenomic replicons was assayed. The activities of the **M2-1** protein phosphorylation mutants in transcriptional antitermination were tested over a range of concns. and were found to be substantially inhibited at all concns. The data show that phosphorylation is important for the **M2-1** protein function in transcription. However, mutation of the **M2-1** phosphorylation sites did not interfere with the ability of the **M2-1** protein to interact with the N protein in transfected cells. The interaction of the **M2-1** and N proteins in contranfecting cells was found to be sensitive to RNase A, indicating that the **M2-1**-N protein interaction was mediated via RNA. Furthermore, the **M2-1** protein was shown to bind monocistronic and polycistronic RS virus mRNAs during infection.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 12 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 8
ACCESSION NUMBER: 2001:845555 CAPLUS
DOCUMENT NUMBER: 136:335929
TITLE: Rescue of bovine **respiratory syncytial virus** from cloned cDNA: entire genome sequence of BRSV strain A51908
AUTHOR(S): Yunus, Abdul S.; Khatrar, Sunil K.; Collins, Peter L.; Samal, Siba K.
CORPORATE SOURCE: Virginia-Maryland Regional College of Veterinary Medicine, University of Maryland, College Park, MD, 20742, USA
SOURCE: Virus Genes (2001), 23(2), 157-164
CODEN: VIGEET; ISSN: 0920-8569
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Infectious bovine **respiratory syncytial virus** (BRSV) was produced by intracellular co-expression of five plasmid born cDNAs, each under the control of a T7 RNA polymerase promoter. These sep. encoded a full-length, genetically-marked copy of BRSV antigenome along with either BRSV or human **respiratory syncytial virus** (HRSV) support plasmids, which express N, P, L and **M2-1** proteins. HEp2 cells were used in transfection and recombinant vaccinia virus (MVA-T7) provided T7 RNA polymerase to drive the transcription. The recovery of recombinant BRSV (rBRSV) was confirmed by immunol. staining of plaques, restriction enzyme digestion and

nucleotide sequencing of PCR fragments carrying the genetic markers from the rescued virus. The rBRSV was indistinguishable from its parental wild-type virus in its growth characteristics in cell culture. The present work has completed the entire genome sequence of BRSV strain A51908 (15,140nt) and has also identified changes in sequence and growth characteristics in cell culture from the original BRSV strain A51908 lab. isolate.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 13 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 11

ACCESSION NUMBER: 2000:569139 CAPLUS

DOCUMENT NUMBER: 134:14435

TITLE: Domains of human **respiratory syncytial virus** P protein essential for homodimerization and for binding to N and NS1 protein

AUTHOR(S): Hengst, Ulrich; Kiefer, Paul

CORPORATE SOURCE: Institut fur Hygiene und Med. Mikrobiologie, Abteilung fur Virologie, Medizinische Fakultat, Ruhr-Universitat Bochum, Bochum, D-44780, Germany

SOURCE: Virus Genes (2000), 20(3), 221-225

CODEN: VIGEET; ISSN: 0920-8569

PUBLISHER: Kluwer Academic Publishers

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In this report the authors used the two-hybrid technique to test for binding among human **respiratory syncytial virus** (HRSV) proteins involved in the control of viral replication. Besides the expected pos. interactions for the nucleoprotein (N) with itself and the phosphoprotein (P), our results also demonstrated P-P interaction and P-NS1 binding. However, no interactions have been detected for the matrix protein M, the **M2-1** and the **M2-2** protein neither with each other nor in combination with the phosphoprotein P, the nucleoprotein N or the non-structural protein NS1. While the N-P interaction was abolished by N- and C-terminal deletions of both partners, C-terminal deletion mutants of P were still able to form homodimers. In contrast, the C-terminal region of P turned out to be essential for binding of NS1. N-N interaction was disrupted by any of the N- and C-terminal deletions.

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 14 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 12

ACCESSION NUMBER: 2000:4833 CAPLUS

DOCUMENT NUMBER: 132:163371

TITLE: **Respiratory syncytial virus** that lacks open reading frame 2 of the M2 gene (M2-2) has altered growth characteristics and is attenuated in rodents

AUTHOR(S): Jin, Hong; Cheng, Xing; Zhou, Helen Z. Y.; Li, Shengqiang; Seddiqui, Adam

CORPORATE SOURCE: Aviron, Mountain View, CA, 94043, USA

SOURCE: Journal of Virology (2000), 74(1), 74-82

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The M2 gene of **respiratory syncytial virus** (RSV) encodes two putative proteins: **M2-1** and **M2-2**; both are believed to be involved in the RNA transcription or replication

process. To understand the function of the M2-2 protein in virus replication, the authors deleted the majority of the M2-2 open reading frame from an infectious cDNA clone derived from the human RSV A2 strain. Transfection of HEp-2 cells with the cDNA clone contg. the M2-2 deletion, together with plasmids that encoded the RSV N, P, and L proteins, produced a recombinant RSV that lacked the M2-2 protein (rA2.DELTA.M2-2). Recombinant virus rA2.DELTA.M2-2 was recovered and characterized. The levels of viral mRNA expression for 10 RSV genes examd. were unchanged in cells infected with rA2.DELTA.M2-2, except that a shorter M2 mRNA was detected. However, the ratio of viral genomic or antigenomic RNA to mRNA was reduced in rA2.DELTA.M2-2-infected cells. By use of an antibody directed against the bacterially expressed M2-2 protein, the putative M2-2 protein was detected in cells infected with wild-type RSV but not in cells infected with rA2.DELTA.M2-2. rA2.DELTA.M2-2 displayed a small-plaque morphol. and grew much more slowly than wild-type RSV in HEp-2 cells. In infected Vero cells, rA2.DELTA.M2-2 exhibited very large syncytium formation compared to that of wild-type recombinant RSV. rA2.DELTA.M2-2 appeared to be a host range mutant, since it replicated poorly in HEp-2, HeLa, and MRC5 cells but replicated efficiently in Vero and LLC-MK2 cells. Replication of rA2.DELTA.M2-2 in the upper and lower respiratory tracts of mice and cotton rats was highly restricted. Despite its attenuated replication in rodents, rA2.DELTA.M2-2 was able to provide protection against challenge with wild-type RSV A2. The genotype and phenotype of the M2-2 deletion mutant were stably maintained after extensive in vitro passages. The attenuated phenotype of rA2.DELTA.M2-2 suggested that rA2.DELTA.M2-2 may be a potential candidate for use as a live attenuated vaccine.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 14

ACCESSION NUMBER: 1998:810729 CAPLUS

DOCUMENT NUMBER: 130:150718

TITLE: Altered growth characteristics of recombinant **respiratory syncytial**

viruses which do not produce NS2 protein

AUTHOR(S): Teng, Michael N.; Collins, Peter L.

CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, 20892-0720, USA

SOURCE: Journal of Virology (1999), 73(1), 466-473

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The second gene in the 3'-to-5' gene order in **respiratory syncytial virus** (RSV) encodes the nonstructural protein

NS2, for which there is no assigned function. To study the function of NS2, we have used a recently developed reverse genetics system to ablate expression of NS2 in recombinant RSV. A full-length cDNA copy of the antigenome of RSV A2 strain under the control of a T7 promoter was modified by introduction of tandem termination codons within the NS2 open reading frame (NS2stop) or by deletion of the entire NS2 gene (.DELTA.NS2). The NS2 knockout antigenomic cDNAs were cotransfected with plasmids encoding the N, P, L, and **M2-1** proteins of RSV, each controlled by the T7 promoter, into cells infected with a vaccinia virus recombinant expressing T7 RNA polymerase. Recombinant NS2stop and .DELTA.NS2 RSVs were recovered and characterized. Both types of NS2 knockout virus displayed pinpoint plaque morphol. and grew more slowly than wild-type RSV. The expression of monocistronic mRNAs for the five genes examd. (NS1, NS2, N, F, and L) was unchanged in cells infected

with either type of NS2 knockout virus, except that no NS2 mRNA was detected with the .DELTA.NS2 virus. Synthesis of readthrough mRNAs was affected only for the .DELTA.NS2 virus, where the NS1-NS2, NS2-N, and NS1-NS2-N mRNAs were replaced with the predicted novel NS1-N mRNA. Upon passage, the NS2stop virus stock rapidly developed revertants which expressed NS2 protein and grew with similar plaque morphol. and kinetics wild-type RSV. Sequence anal. confirmed that the termination codons had reverted to sense, albeit not the wild-type assignments, and provided evidence consistent with biased hypermutation. No revertants were recovered from recombinant .DELTA.NS2 RSV. These results show that the NS2 protein is not essential for RSV replication, although its presence greatly improves virus growth in cell culture. The attenuated phenotype of these mutant viruses, coupled with the expected genetic stability assocd. with gene deletions, suggests that the .DELTA.NS2 RSV is a candidate for vaccine development.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 15

ACCESSION NUMBER: 1999:409828 CAPLUS

DOCUMENT NUMBER: 131:209780

TITLE: Support Plasmids and Support Proteins Required for Recovery of Recombinant **Respiratory Syncytial Virus**

AUTHOR(S): Collins, Peter L.; Camargo, Ena; Hill, Myron G.

CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, 20892-0720, USA

SOURCE: Virology (1999), 259(2), 251-255

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Respiratory syncytial virus** (RSV) can be recovered from plasmids that sep. encode antigenomic RNA and the N, P, L, and **M2-1** proteins of the nucleocapsid. However, in a recent study the inclusion of a sep. **M2-1** expression plasmid was found to be unnecessary (H. Jin, D. Clarke, H. Zhou, X. Cheng, K. Coelingh, M. Bryant, and S. Li, Virol. 1998, 251, 206-214). This suggested that the **M2-1** protein, which is a transcription antitermination factor, is not required to reconstitute the min. unit of infectivity, namely a nucleocapsid fully functional for viral transcription and RNA replication. Here we show that the antigenomic plasmid is remarkably efficient as a substitute for an **M2-1** expression plasmid in supporting processive transcription by an RSV minigenome. Thus, the simple expedient of omitting an expression plasmid is invalid for evaluating recovery requirements. The issue of the requirement of **M2-1** for the recovery of infectious RSV is discussed. (c) 1999 Academic Press.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 16

ACCESSION NUMBER: 1998:395624 CAPLUS

DOCUMENT NUMBER: 129:119939

TITLE: Identification of the **respiratory syncytial virus** proteins required for formation and passage of helper-dependent infectious particles

AUTHOR(S): Teng, Michael N.; Collins, Peter L.

CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute

of Allergy and Infectious Diseases, Bethesda, MD,
20892-0720, USA

SOURCE: Journal of Virology (1998), 72(7), 5707-5716
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors developed a system to identify the viral proteins required for the packaging and passage of human **respiratory syncytial virus** (RSV) by reconstructing these events with cDNA-encoded components. Plasmids encoding individual RSV proteins, each under the control of a T7 promoter, were cotransfected in various combinations together with a plasmid contg. a minigenome into cells infected with a vaccinia virus recombinant expressing T7 RNA polymerase. Supernatants from these cells were passaged onto fresh cells which were then superinfected with RSV. Functional reconstitution of RSV-specific packaging and passage was detected by expression of the reporter gene carried on the minigenome. As expected, the four nucleocapsid proteins N, P, L, and **M2-1** failed to direct packaging and passage of the minigenome. Passage was achieved by further addn. of plasmids expressing three membrane-assocd. proteins, M, G, and F; inclusion of the fourth envelope-assocd. protein, SH, did not alter passage efficiency. Passage was reduced 10-20-fold by omission of G and was abrogated by omission of either M or F. Coexpression of the nonstructural NS1 or NS2 protein had little effect on packaging and passage except through indirect effects on RNA synthesis in the initial transfection. The **M2-1** transcription elongation factor was not required for the generation of passage-competent particles. However, addn. of increasing quantities of **M2-1** to the transfection mediated a dose-dependent inhibition of passage which was alleviated by coexpression of the putative neg. regulatory factor M2-2. Omission of the L plasmid reduced passage 10- to 20-fold, most likely due to reduced availability of encapsidated minigenomes for packaging. However, the residual level of passage indicated that neither L protein nor the process of RSV-specific RNA synthesis is required for the prodn. and passage of particles. Omission of N or P from the transfection abrogated passage. Thus, the min. RSV protein requirements for packaging and passaging a minigenome are N, P, M, and F, although the efficiency is greatly increased by addn. of L and G.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 18

ACCESSION NUMBER: 1998:753673 CAPLUS

DOCUMENT NUMBER: 130:120171

TITLE: Recombinant human **respiratory syncytial virus** (RSV) from cDNA and construction of subgroup A and B chimeric RSV

AUTHOR(S): Jin, Hong; Clarke, David; Zhou, Helen Z.-Y.; Cheng, Xing; Coelingh, Kathleen; Bryant, Martin; Li, Shengqiang

CORPORATE SOURCE: Aviron, Mountain View, CA, 94043, USA

SOURCE: Virology (1998), 251(1), 206-214
CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Infectious human **respiratory syncytial virus** (RSV) was produced from a cDNA clone that contains 15,222 nucleotides of RSV genome derived from the A2 strain of subgroup A. Recovery of infectious RSV from cDNA required cotransfection of only three expression

plasmids encoding the nucleoprotein (N), the phosphoprotein (P), and the major polymerase protein (L). Inclusion of the **M2-1** plasmid was not required in the transfection reaction and if included did not significantly increase the rescue efficiency. However, a single nucleotide substitution in the RSV leader region (C to G at position 4 in the antigenomic sense), greatly increased the amt. of infectious virus recovered from cDNA. A recombinant RSVA2 virus that expresses an addnl. structural G protein derived from a subgroup B RSV was also obtained. Both A2 and B strain G glycoproteins were expressed in cells infected with the chimeric RSV. A chimeric RSV that expresses a heterologous subgroup antigen in a live attenuated vaccine candidate may be important for prevention of diseases assocd. with both RSV subgroup A and subgroup B infection. (c) 1998 Academic Press.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:429041 CAPLUS

DOCUMENT NUMBER: 137:19372

TITLE: Recombinant **respiratory syncytial virus** comprising deleted or attenuated accessory gene(s) for use as vaccines and vaccine expression systems

INVENTOR(S): Jin, Hong; Tang, Roderick; Li, Shengqiang; Bryant, Martin

PATENT ASSIGNEE(S): Aviron, Inc., USA

SOURCE: PCT Int. Appl., 150 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002044334	A2	20020606	WO 2001-US44819	20011128
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

AU 2002036522 A5 20020611 AU 2002-36522 20011128

PRIORITY APPLN. INFO.: US 2000-724416 A 20001128

WO 2001-US44819 W 20011128

AB The present invention relates to genetically engineered recombinant **respiratory syncytial viruses** and viral vectors which contain deletions of various viral accessory gene(s) either singly or in combination. In accordance with the present invention, the recombinant respiratory syncytial viral vectors and viruses are engineered to contain complete deletions of the M2-2, NS1, NS2, or SH viral accessory genes or various combinations thereof. In addn., the present invention relates to the attenuation of **respiratory syncytial virus** by mutagenesis of the **M2-1** gene.

L60 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2003:8769 CAPLUS

TITLE: Effect of RSV **M2-1** gene expression

AUTHOR(S): on the growth of human lung adenocarcinoma PAa
Chen, Hangwei; Du, Yuguo; Xin, Qinghong; Li, Jicheng
CORPORATE SOURCE: General Hospital of Beijing Command, Beijing, 100700,
Peop. Rep. China
SOURCE: Jiefangjun Yixue Zazhi (2002), 27(7), 585-587
CODEN: CFCHBN; ISSN: 0577-7402
PUBLISHER: Jenminjun Chubanshe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The effect of RSV **M2-1** gene expression on the growth of human lung adenocarcinoma PAa cell line was studied. The recombinant RSV **M2-1** gene eukaryotic plasmid PXJ-41/**M2-1** was transfected to the human lung adenocarcinoma PAa cell line. Expression of the **M2-1** gene was examd. by RT-PCR and Western blot. The growth of the human lung adenocarcinoma PAa cell was obsd. by MTT curve, flow cytometry, the capacity of inherence, colony-forming units and inoculation of nude mouse. The results showed that (1) The desired fragments of **M2-1** gene were digested by restriction enzyme and RT-PCR, resp. (2) The bands of **M2-1** gene protein were found by Western blot. (3) The ratio of transfected PAa cells in S phase of cell cycle was decreased, but increased in G2/M phase. Colony-forming efficiency and units of the transfected PAa cell were increased, but the capacity of its inherence was decreased. (4) Tumorigenesis time of the transfected PAa cell in nude mouse was delayed, but its growing speed was increased. The tumor tissue transformed to some extent from adenocarcinoma to squamous carcinoma was found in the nude mouse. It is suggested that the **M2-1** gene may promote the growth of PAa cells, which may implicate some relationship between RSV and lung cancer.

L60 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:669124 CAPLUS
DOCUMENT NUMBER: 136:335760
TITLE: Construction and expression of human
respiratory syncytial virus
M2-1 gene plasmid in prokaryotic cells

AUTHOR(S): Xin, Qinghong; Li, Jicheng; Chen, Hangwei; You, Lanhua
CORPORATE SOURCE: Department of Respiratory Diseases, General Hospital
of Beijing Military Command, Beijing, 100700, Peop.
Rep. China
SOURCE: Di-San Junyi Daxue Xuebao (2001), 23(7), 827-829
CODEN: DYXUE8; ISSN: 1000-5404
PUBLISHER: Di-San Junyi Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The human **respiratory syncytial virus** (hRSV) **M2-1** gene vector was constructed and its expression in prokaryotic cells was studied. Total RNA of hRSV-infected Hep-2 cells was extd. and **M2-1** gene fragment was amplified by RT-PCR. The amplified **M2-1** fragment and pGEX-2T were Digested by EcoR I and Xho I, ligated, and transfected into prokaryotic cells. The pos. cloned bacteria were identified by PCR and enzyme digestion and its expression was induced by IPTG. The results showed that the prokaryotic expression plasmid contg. hRSV-**M2-1** gene was constructed and target gene was expressed in objective cells.

L60 ANSWER 22 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:608951 CAPLUS
DOCUMENT NUMBER: 133:202979
TITLE: Methods of screening for antiviral compounds directed

to **respiratory syncytial virus** based on function of **M2-1** protein
INVENTOR(S): Wertz, Gail W.; Hardy, Richard W.; Douglas, Joanne T.
PATENT ASSIGNEE(S): Uab Research Foundation, USA
SOURCE: PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000050646	A1	20000831	WO 2000-US4552	20000223
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6376171	B1	20020423	US 2000-511023	20000223
US 2002119446	A1	20020829	US 2002-127318	20020422
PRIORITY APPLN. INFO.:			US 1999-122251P	P 19990226
			US 2000-511023	A3 20000223

AB The present invention demonstrates that the **M2-1** protein of **respiratory syncytial virus** has a conserved Cys3-His1 motif known to bind zinc ions in other proteins and that mutations of the predicted zinc coordinating residues in the Cys3-His1 motif affect the transcriptional antitermination activity of **M2-1**, its ability to interact with nucleocapsid protein, and the phosphorylation state of **M2-1**. This invention clearly demonstrates the requirement for conservation of the Cys3-His1 motif in order to maintain the functional integrity of the **M2-1** protein. Therefore, the present invention provides for methods of designing and screening compds. for antiviral activity towards **respiratory syncytial virus** based upon the loss of function of the **M2-1** protein.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 23 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:398751 CAPLUS

DOCUMENT NUMBER: 131:180735

TITLE: Role of the **M2-1** transcription antitermination protein of **respiratory syncytial virus** in sequential transcription

AUTHOR(S): Fearn, Rachel; Collins, Peter L.

CORPORATE SOURCE: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, 20892-0720, USA

SOURCE: Journal of Virology (1999), 73(7), 5852-5864

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **M2-1** protein of human **respiratory**

syncytial virus (RSV) is a transcription antitermination factor that is important for the efficient synthesis of full-length mRNAs as well as for the synthesis of polycistronic read-through mRNAs, which are characteristic of nonsegmented neg.-strand RNA viruses. The contributions of these effects to RSV sequential transcription were investigated with minigenomes which contained one to five genes which were either foreign marker genes or authentic RSV genes. When evaluated on a promoter-proximal gene, the effect of **M2-1** on the synthesis of full-length mRNA was much greater for a long (1212- or 1780-nucleotide) gene (up to a 615-fold increase) than for a short (274-nucleotide) gene (less than a 2-fold increase). This was independent of whether the gene contained non-RSV or RSV-specific sequence. Once the polymerase had terminated prematurely, it was unable to reinitiate at a downstream gene. These studies also confirmed that **M2-1** enhances the synthesis of polycistronic mRNAs and that the magnitude of this effect varied greatly among different naturally occurring gene junctions. The synthesis of polycistronic mRNAs, which presumably involves antitermination at the gene-end signal, required a higher level of **M2-1** than did the synthesis of the corresponding monocistronic mRNAs. **M2-1** did not have a comparable antitermination effect at the junction between the leader region and the first gene. In a minigenome contg. the NS1 and NS2 genes in their authentic sequence context, synthesis of full-length NS1 and NS2 mRNAs in the absence of **M2-1** was remarkably high (36 and 57%, resp., of the max. levels obsd. in the presence of **M2-1**). In contrast, synthesis of mRNA from addnl. downstream genes was highly dependent on **M2-1**. Thus, RSV has the potential for two transcription programs: one in the absence of **M2-1**, in which only the NS1 and NS2 genes are transcribed, and one in the presence of **M2-1**, in which sequential transcription of the complete genome occurs. The dependence on **M2-1** for transcription was greater for a gene in the fifth position from the promoter than for one in the third position. This indicates that under conditions where **M2-1** is limiting, its concn. affects the gradient of transcription. Although **M2-1** was found to have profound effects on transcription, it had no effect on replication of any minigenome tested, suggesting that it is not an active participant in RNA replication or regulation of RNA replication. Finally, since a permissive RSV infection is marked by a gradual increase in the intracellular accumulation of viral proteins including **M2-1**, we examd. the relative abundances of various mRNAs during RSV infection for evidence of temporal regulation of transcription. None was found, implying that the availability of **M2-1** during a permissive infection is sufficient at all times such that its concn. does not mediate temporal regulation of gene transcription.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 24 OF 24 WPIDS (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: 2001-103088 [11] WPIDS
DOC. NO. CPI: C2001-030283
TITLE: Isolated chimeric human-bovine **respiratory syncytial virus** (RSV), useful in an attenuated **vaccine** to elicits an immune response against either or both human RSV A or RSV B.
DERWENT CLASS: B04 D16
INVENTOR(S): BUCHHOLZ, U; COLLINS, P L; KREMPLE, C D; MURPHI, B R; WHITEHEAD, S S; KREMPLE, C D; MURPHY, B R
PATENT ASSIGNEE(S): (USSH) US DEPT HEALTH & HUMAN SERVICES
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001004335	A2	20010118	(200111)*	EN	148
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000056415	A	20010130	(200127)		
BR 2000013195	A	20020723	(200257)		
EP 1287152	A2	20030305	(200319)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001004335	A2	WO 2000-US17755	20000623
AU 2000056415	A	AU 2000-56415	20000623
BR 2000013195	A	BR 2000-13195	20000623
		WO 2000-US17755	20000623
EP 1287152	A2	EP 2000-941756	20000624
		WO 2000-US17755	20000624

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000056415	A Based on	WO 200104335
BR 2000013195	A Based on	WO 200104335
EP 1287152	A2 Based on	WO 200104335

PRIORITY APPLN. INFO: US 1999-143132P 19990709

AB WO 200104335 A UPAB: 20030214

NOVELTY - An isolated chimeric human-bovine **respiratory syncytial virus** (RSV) that is infectious and attenuated in humans, is new.

DETAILED DESCRIPTION - An isolated chimeric human-bovine **respiratory syncytial virus** (RSV) that is infectious and attenuated in humans, is new.

The virus comprises a major nucleocapsid protein (N), a nucleocapsid phosphoprotein (P), a large polymerase protein (L), a RNA polymerase elongation factor, and a partial or complete RSV background genome, or antigenome of a human RSV or bovine RSV, combined with one or more heterologous gene(s) or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome or antigenome.

INDEPENDENT CLAIMS are also included for the following:

(1) a method (M1) for stimulating the immune system of an individual to induce protection against RSV, comprising administering an immunologically sufficient amount of the chimeric RSV;

(2) an isolated polynucleotide comprising a chimeric RSV genome or antigenome which includes a partial or complete RSV background genome or antigenome of a human or bovine RSV combined with one or more heterologous gene(s) or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome or antigenome; and

(3) a method (M2) for producing an infectious attenuated chimeric RSV particle from one or more isolated polynucleotide molecules encoding the RSV, comprising expressing RSV N, P, L and RNA polymerase elongation factor proteins, and an expression vector comprising the polynucleotide of

(2) in a cell or cell-free lysate.

ACTIVITY - Antiviral.

Young chimpanzees which were determined to be seronegative for human RSV were inoculated by both the intranasal and intratracheal routes with a dose of 107 pfu (plaque forming units) per ml of rBRSV or rBRSV/A2 at each site. Each virus was administered to two chimpanzees. Following inoculation of the virus, nasopharyngeal swab samples were taken daily on days 1-10 and 12, and tracheal lavage samples were taken on days 2, 5, 6, 8 and 12. Specimens were frozen and RSV titers were measured later by plaque assay on HEP-2 cells. The amount of rhinorrhea, a measure of upper respiratory tract illness, was estimated daily and assigned a score of 0-4 (0=none, 1= trace, 2= mild, 3= moderate, 4= severe). The results were compared to historic controls of animals which had received:

(i) 104 pfu of recombinant human RSV strain A2 wild type virus per site (Whitehead, et al., J. Virol. 72:4467-4471, 1998) or

(ii) 105 pfu of the live-attenuated rA2cp28/404 strain A2 **vaccine** candidate per site (Whitehead, et al., J. Virol. 73:343 8-3442, 1999), administered by the same routes.

Wild type human RSV was highly permissive in seronegative chimpanzees, and in this exercise replicated to peak mean titers of more than 4.5 log₁₀ pfu per ml of nasal swab or tracheal lavage sample. The peak rhinorrhea score was 2.5. The live- attenuated **vaccine** candidate rA2cp248/404 (see, e.g., U.S. Patent No. 5,993,824, issued November 30, 1999; International Publication No. WO 98102530; Collins, et al., Proc Natl. Acad. Sci. USA 92:11563-11567, 1995; Whitehead, et al., Virology 247:232-239, 1998) replicated to mean peak titers of 2.5 and 1.4 log₁₀ pfu per ml of swab/lavage in the upper and lower respiratory tracts, respectively, and had a peak rhinorrhea score of 0.8. In contrast, there was no detectable replication of recombinant bovine (rBRSV) in either the upper or lower respiratory tracts and no evidence of disease. Thus, even when administered at 100-1000 times the dose of human RSV, rBRSV was highly restricted for replication in chimpanzees. The rBRSV/A2 chimera exhibited replication over several days in both the upper and lower respiratory tract.

The shedding was not detected until day 3 or 5 indicates that it was not carryover from the inoculation, as does the length of time over which virus was recovered. The titers were much lower than observed for wild type human RSV and moderately lower than observed for the rA2cp248/404 **vaccine** candidate. These results indicate that the chimeric virus was highly attenuated. Thus, replacement of the G and F glycoprotein genes of rBRSV with their human RSV counterparts, which transferred the major antigenic determinants, confers improved growth in chimpanzees while other bovine RSV genes contribute to a highly attenuated phenotype.

MECHANISM OF ACTION - Immunostimulant; Anti-RSV **vaccine**.

USE - The chimeric RSV is useful in an attenuated **vaccine** to elicits an immune response against either or both human RSV A or RSV B (claimed).

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FILE 'HOME' ENTERED AT 18:09:27 ON 15 APR 2003